DEPARTMENT OF CLINICAL MICROBIOLOGY
CHRISTIAN MEDICAL COLLEGE
Ida Scudder Road, Vellore - 632 004, India.

Dr. V. Balaji, MD, PhD, FRCP, Professor
Dr. Joy S Michael, MD, FRCPath, Professor
Dr. John A Jude Prakash, MD, Professor
Dr. Rani Diana Sahni, MD, DTM&H Professor
Dr. Binesh Lal Y, MD, Professor

Dr. Beula Subashini P, MD, Ph.D, Associate Professor
Dr. Marilyn M Ninan, DCP, MD, Associate Professor
Dr. Susmitha Karunasree P, MD, DTM&H, Assistant Professor
Dr. S. Dhanalakshmi, MD, Assistant Professor
Dr. Lydia Jennifer S, MD, Assistant Professor

Genomics-informed outbreak investigations of drug resistant *P. aeruginosa* associated with Artificial Tears

As of May 15, 2023, the Centers for Disease Control and Prevention (CDC) has disclosed a widespread outbreak of an extensively drug-resistant strain of *Pseudomonas aeruginosa* (CDC, 2023; https://www.cdc.gov/hai/outbreaks/crpa-artificial-tears.html). The outbreak has affected 81 patients across 18 states in the United States, resulting in four fatalities. Additionally, there have been reports of local transmission within healthcare facilities in USA (Sundermann et al., 2023). This outbreak is associated to various types of infections, especially eye infections that reported to be linked to the use of EzriCare artificial tears, as individuals who used this product developed keratitis (Shoji et al., 2023). The outbreak strain belonged to sequence type (ST) 1203 and contains resistance genes bla_{VIM-80} and bla_{GES-9} that confer resistance to carbapenems. According to publicly available genome data in databases, carbapenem resistant *P. aeruginosa* (CRPA) carrying both VIM-80 and GES-9 has not been previously identified (CDC, 2023).

The suspected source of the VIM-GES-CRPA outbreak is believed to be 'EzriCare Artificial Tears' eyedrops (CDC, 2023). This suspicion arises from the detection of the same strain in opened EzriCare bottles from multiple lots, as confirmed by both the CDC and FDA (FDA, 2023; https://www.fda.gov/drugs/drug-safety-and-availability/fda-warns-consumers-not-purchase-or-use-ezricare-artificial-tears-due-potential-contamination). However, the testing conducted by the FDA on unopened bottles of EzriCare Artificial Tears was unable to identify the outbreak strain, despite reports of other bacterial contamination. As of now, these products have been voluntarily recalled by their manufacturer (Global Pharma, Chennai, Tamil Nadu,

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India). Given the challenges in precisely identifying the source of the VIM-GES-CRPA outbreak, the CDC has urged healthcare facilities to report potential cases with similar strains (CDC, 2023). Additionally, the CDC has offered genome sequences of representative outbreak isolates to facilitate comparisons and enhance the understanding of the situation. Subsequent surveillance studies in U.S. hospitals, including CDC-HAI-Seq (PRJNA288601) and EDS-HAT (PRJNA475751), have successfully identified cases of VIM-GES-CRPA associated with the outbreak. All the outbreak associated isolates (clinical, CDC-HAI, & EDS-HAT) belong to ST1203. The VIM-GES-CRPA ST1203 has been reported previously from UK, Belgium, Kenya, Nigeria, Australia, Netherlands, Colombia, and India (Sundermann et al., 2023). Christian Medical College (CMC), Vellore has given responsibility by Indian council of medical research (ICMR) to conduct a nation-wide surveillance in drug resistant *P. aeruginosa*, to serve as a reference laboratory. The research team under Dr. Balaji Veeraraghavan has generated baseline data on molecular resistance mechanisms for most clinical pathogens in partnership with multiple participating sites across India. Here, we have attempted to source track the presence of VIM-GES-CRPA strains from Indian clinical settings by phylogenetic analysis and comparative genome analysis.

For comparative analysis, contextual clinical isolates belonging ST1203 from ICMR AMRSN surveillance network (2021 – 2022) was revived from the Archive at the Dept. of Clinical Microbiology, Christian Medical College, Vellore. Genomic DNA was extracted from all isolates and sequenced on the Illumina platform. Additionally, *P. aeruginosa* ST1203 genomes, were downloaded from the National Center for Biotechnology Information (NCBI) database and Psedomonas.com on 27th November 2023. Phylogenetic tree was constructed using IQTree2 (K3P + ASC model) from a core genome alignment generated with snippy aligned against reference genome PA01 (NC_002516). The genetic background of bla_{GES-9} belonging to outbreak strain (CP121766) and Indian isolate (GCF_016836545.1) was compared using BLAST and EasyFig (https://mjsull.github.io/Easyfig/).

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Phylogenetic analysis revealed close relatedness of CDC-HAI-Seq (n=97) and EDS-HAT (n=3) to the representative outbreak isolates provided by CDC and FDA (n=18). All 118 outbreak associated isolates carried both bla_{VIM-80} and bla_{GES-9} resistance genes and were separated by 0-4 SNPs within the highlighted cluster (**Figure 1**). The closest isolate (contextual isolate from India) carried bla_{GES-9} but devoid of bla_{VIM-80} and separated by 32 SNPs from the outbreak cluster. Similarly, another Indian isolate (Historical isolate from an eye infection) carried bla_{GES-9} and bla_{VIM-80} were 23 SNPs away from the outbreak strains. Interestingly, the Indian contextual isolates from ICMR AMRSN network collection were separated by 70 SNPs and carried carbapenemase genes such as bla_{NDM} and bla_{DIM}. Overall, the VIM-GES-CRPA outbreak cluster is at least 32 SNPs distant from Indian contextual isolates and historical and suggest a possible independent evolution.

We have also compared the genetic background of bla_{GES-9} between the outbreak strains and Indian historical strain to understand whether the chromosomal integration of GES carrying transposon is a same or different evolutionary event. As observed in **Figure 2**, the transposon carrying bla_{GES-9} has been integrated in different regions in comparison to the reference genome (PA01). Moreover, the genetic background of bla_{GES-9} carrying transposon is diverse in both Indian and outbreak isolates. Indian isolates lack ISVsa3/IS91 (Tnp307), which carry bla_{GES-9} in CDC-HAI-Seq samples. This confirms the independent origin of bla_{GES-9} among these strains and further diversity evolutionary trajectory of Indian and outbreak strains.

Conclusion

Presently, there is a lack of genomic evidence substantiating the definitive origin of VIM-GES-CRPA from India. Analysis of CRPA isolates archived at CMC Vellore from 2018 onwards indicates a rare co-occurrence of bla_{GES-9} and bla_{VIM-80}. Moreover, the genetic background of the presently sequenced Indian isolates carrying bla_{GES-9} differs from that of the USA outbreak isolates. The Whole Genome Sequencing (WGS) conducted focused only on a representative subset of CRPA isolates from patients with clinical infections. To conduct a conclusive outbreak investigation, additional WGS data from contextual isolates in India are deemed necessary.

Tel : 91 (0) 416-2282588   Fax : 91 (0) 416-2232103   e-mail : micro@cmcvellore.ac.in

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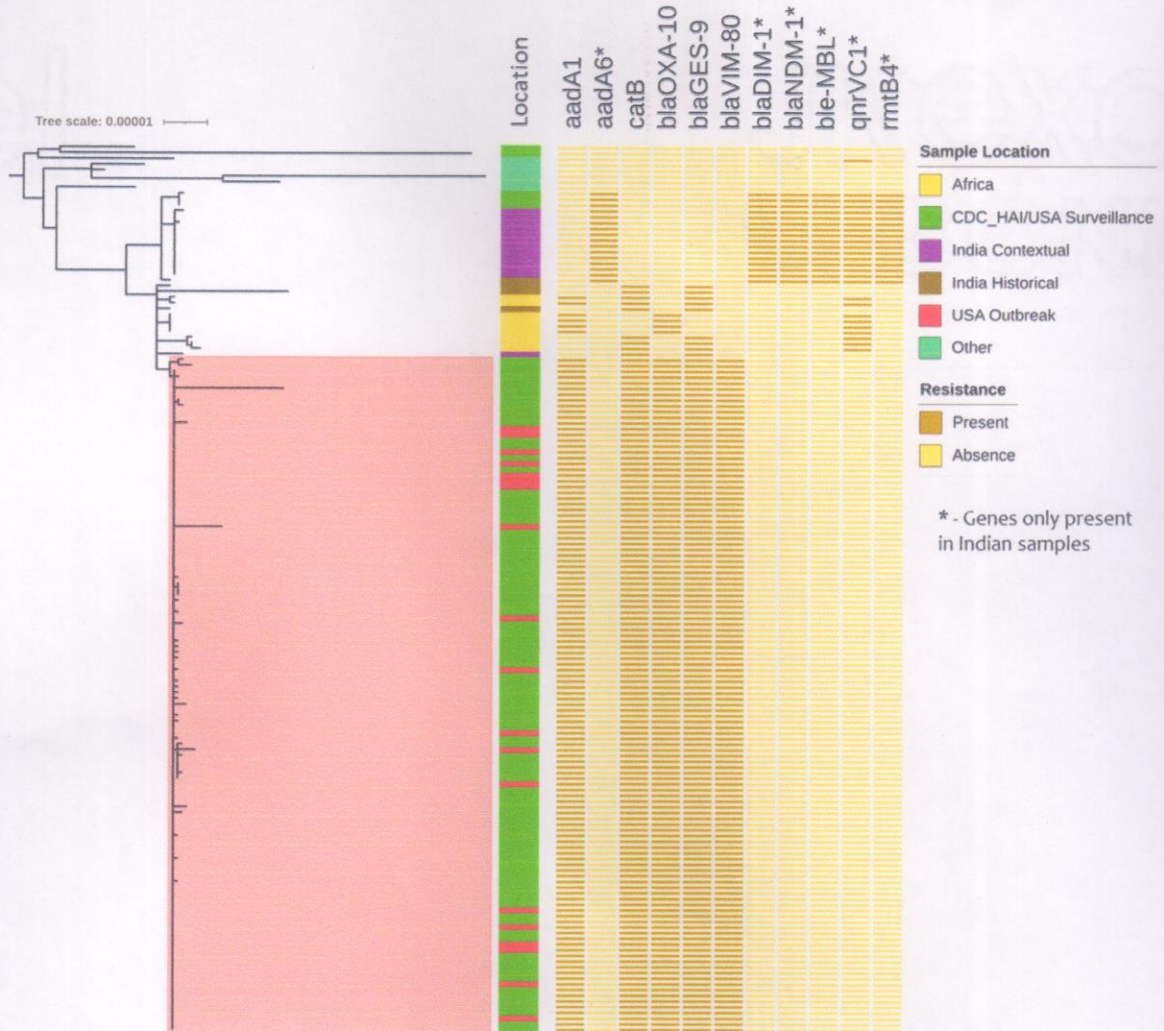**Dr. Lydia Jennifer S,** MD, Assistant Professor

Figure 1: Global phylogeny of publicly available ST1203 *Pseudomonas aeruginosa* genomes.

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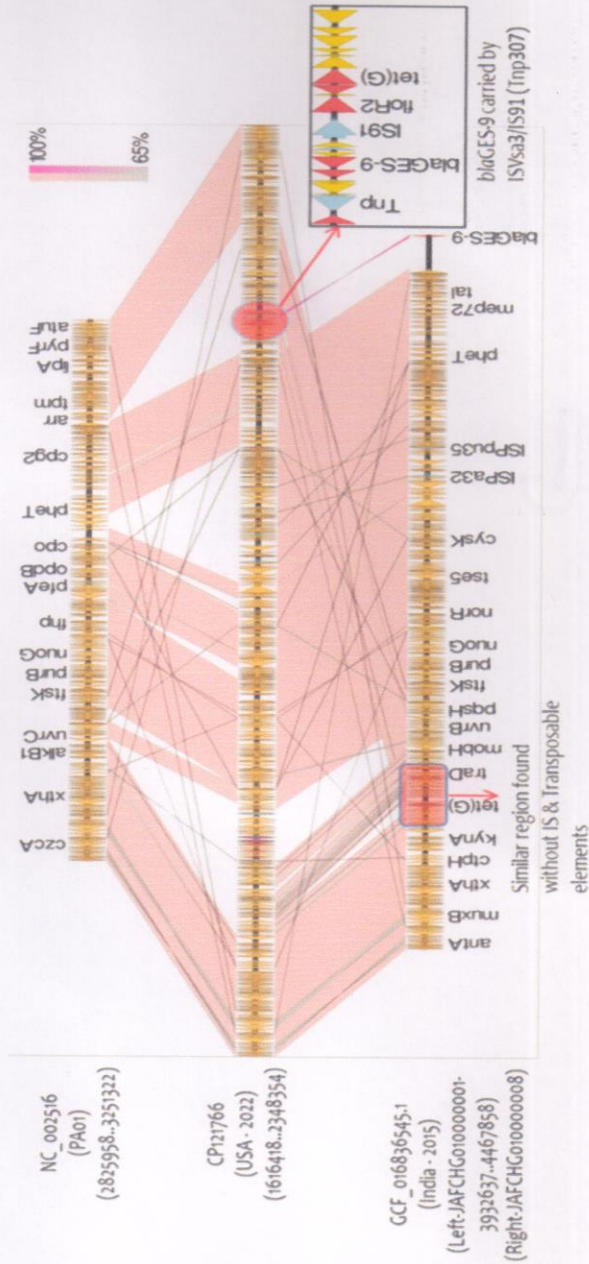**Dr. Lydia Jennifer S,** MD, Assistant Professor

Figure 2: The genomic regions of PA01, CDC-HAI seq, and Indian Historical isolate are compared linearly in Easyfig.

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Dr. V. Balaji
Senior Professor

Dr. Balaji Veeraraghavan MD, Ph.D.,
Professor
Dept. of Clinical Microbiology
Christian Medical College & Hospital
Vellore 632 004 South India